



# Global Molecular Characterization of the Chromate Stress Response in *Shewanella oneidensis* MR-1:

## Identification of a Putative DNA-Binding Response Regulator and Azoreductase Involved in Cr(VI) Detoxification

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### OVERVIEW

*Shewanella oneidensis* MR-1 is a model environmental organism that possesses diverse respiratory capacities, including the ability to reduce soluble Cr(VI) to sparingly soluble, less toxic Cr(III). Effective bioremediation of Cr-contaminated sites requires knowledge of the molecular mechanisms and regulation of heavy metal resistance and biotransformation by dissimilatory metal-reducing bacteria. Towards this goal, our ERSP-funded work is focused on the identification and functional analysis of genes/proteins comprising the response pathways for chromate detoxification and/or reduction. Previous transcriptomic profiling and whole-cell proteomic analyses implicated the involvement of a functionally undefined DNA-binding response regulator (SO2426) and a putative azoreductase (SO3585) in the chromate stress response of MR-1.

Here we describe a detailed functional analysis of SO2426 and SO3585 in order to begin to understand the role of these proteins in the cellular response to chromate. The protein products encoded by genes *so2426* and *so3585* were expressed and detected only in chromate-shocked samples as determined by multidimensional high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Both genes were also highly induced (>46-fold) in MR-1 cells actively reducing chromate based on whole-genome microarray analysis. We have created in-frame deletions of the *so2426* and *so3585* loci in the MR-1 chromosome and have characterized the phenotype of the resulting mutants in the presence of varying concentrations of Cr, Cu, Co, Sr, and H<sub>2</sub>O<sub>2</sub> under aerobic respiratory conditions. Growth studies indicated that the *so2426* deletion mutant was more sensitive to heavy metals compared to the WT reference, and chromate reduction by the *so2426* mutant was impaired significantly. The growth response of the mutant to H<sub>2</sub>O<sub>2</sub> was similar to that of MR-1. To gain insight into the regulon of this response regulator, MR-1 microarrays were used to explore dynamic changes in the WT and *so2426* mutant transcriptomes during chromate stress and reduction.

The *so3585* deletion mutant resembled the WT in terms of growth; however, this mutant was able to reduce chromate at a substantially faster rate compared to the WT strain. Based on its genomic proximity and coregulated expression profile, we predict that SO3585 functions in a complex together with the proteins SO3586 (glyoxalase family) and membrane-associated SO3587 (hypothetical protein). Future studies will include purifying SO3585 to determine whether it can reduce Cr(VI) and whether it interacts with SO3586 and SO3587.

### EXPERIMENTAL

- S. oneidensis* WT and mutant strains were grown in Luria-Bertani (LB) medium in presence or absence of different concentrations of added metal at 30°C under aerobic conditions. Growth was monitored using a Bioscreen C microbiodigital culture system (Growth Curves USA). Cr(VI) reduction was measured spectrophotometrically at wavelength 540 nm using the 1,5-diphenylcarbazide (DPC) method (Park, C. H., M. Keyhan, B. Welting, S. Fendorf, and A. Mattin, 2000. Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase. *Appl. Environ. Microbiol.* 66:1788-1795).
- In-frame deletions of the *so2426* and *so3585* loci (FIG. 1, A and B) in the MR-1 chromosome were created using a *cre-lox*-based recombination system [Dennet V.J., Klappenbach J.K., Patrauchen M.A., Floriano C., Rodriguez J.L., Tsai T.V., Verschuere W., Ellis G., Tiedje J.M. 2006 Genetic and genomic insights into the role of denitrification pathway redundancy in *Burkholderia xenovorans* LB400. *Appl Environ Microbiol.* 72(1):585-591).
- A whole-genome cDNA array containing approx. 95% of the total predicted *S. oneidensis* MR-1 gene content was used to examine global transcriptional changes in response to Cr challenge. Gene expression profiling was performed using six independent microarray experiments (two dye reversal reactions x three biological replicates). Transcripts exhibiting a statistically significant change in expression ( $P < 0.05$ ) and a twofold or greater change in magnitude were further considered.
- Confocal laser scanning microscopy using a Leica TCS SP2 microscope was used to examine the impact of Cr on cell morphology.

### RESULTS

TABLE 1. Transcriptome Profiling under Conditions of Cr(VI) Challenge

ORF	Gene Product	Time=	5 min <sup>a</sup>	30 min <sup>a</sup>	60 min <sup>a</sup>	90 min <sup>a</sup>	3 h <sup>b</sup>	24 h <sup>c</sup>
<i>so2426</i>	DNA-binding response regulator		3.7	3.3	3.5	10.7	46.9	0.6
<i>so3585</i>	azoreductase, putative		5.6	60.9	28.2	30.1	52.6	0.7
<i>so3586</i>	glyoxalase family protein		3.8	26.4	16.1	13.1	14.1	0.5
<i>so3587</i>	hypothetical protein		3.7	17.5	10.4	14.2	3.2	0.9

<sup>a</sup>Relative gene expression (fold induction) 5, 30, 60, and 90 min post-1 mM chromate addition. No Cr(VI) reduction measured.

<sup>b</sup>Relative gene expression (fold induction) 3 h post-0.3 mM chromate addition. Cells actively reducing Cr(VI).

<sup>c</sup>Relative gene expression (fold induction) 24 h post-0.3 mM chromate addition. Complete Cr(VI) reduction.

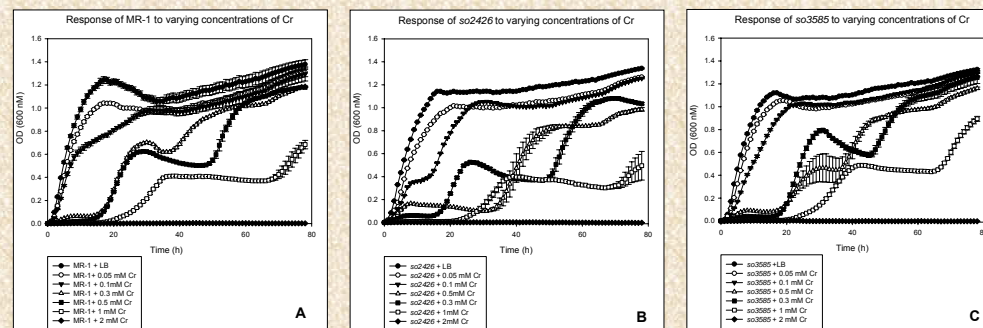


FIG. 2. Growth of MR-1 (A), deletion mutant  $\Delta so2426$  (B), and deletion mutant  $\Delta so3585$  (C) in LB in the absence or presence of different chromate concentrations.

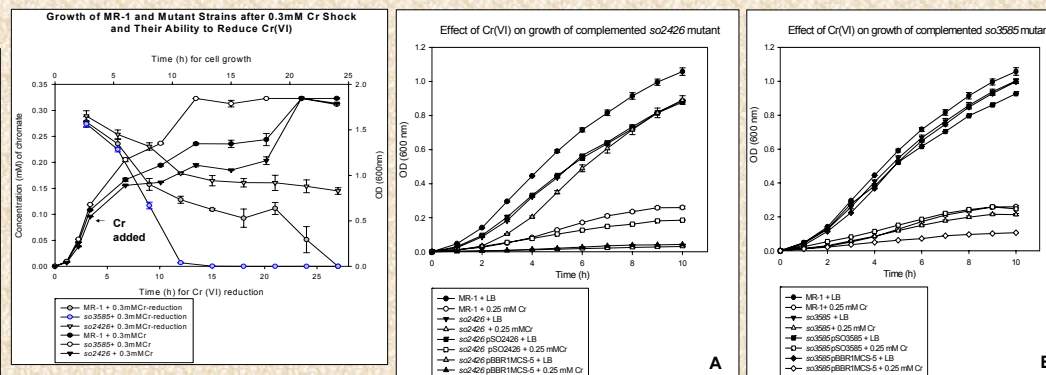


FIG. 3. Growth and Cr(VI) disappearance patterns for *S. oneidensis* MR-1 and mutant strains. No abiotic conversion of chromate was detected in the LB broth-only control (data not shown).

FIG. 4. Restoration of growth phenotype in complemented mutant  $\Delta so2426$  (A) and complemented mutant  $\Delta so3585$  (B) in LB amended with 0.250 mM chromate.

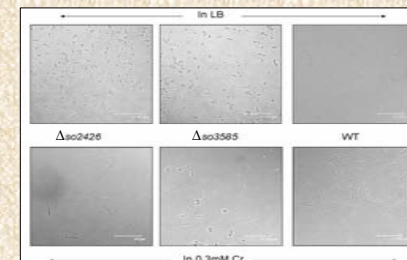


FIG. 5. Scanning confocal micrographs of WT and mutant *S. oneidensis* strains grown in the absence and presence of 0.3 mM chromate. Cells were imaged in brightfield mode.

### SUMMARY

- Based on our previous transcriptomic profiling and whole-cell proteomic analyses of MR-1 under chromate stress conditions, we identified a functionally undefined DNA-binding response regulator (SO2426) and a putative azoreductase (SO3585) as playing potentially important roles in the cellular response to Cr(VI) stress. Both the transcripts and encoded proteins for these genes were found to be up-regulated at high levels under Cr conditions (S.D. Brown, M.R. Thompson, N.C. VerBerkmoes, K. Chourey, M. Shah, J. Zhou, R.L. Hettich, and D.K. Thompson, 2006. Molecular dynamics of the *Shewanella oneidensis* response to chromate stress. *Molecular and Cellular Proteomics*, in press).
- We created MR-1 mutant strains harboring in-frame deletions of the *so2426* and *so3585* loci and characterized the phenotypes of these strains compared to MR-1. Growth studies indicated that the *so2426* deletion mutant was more sensitive to chromate compared to the WT reference, and Cr(VI) reduction by the *so2426* mutant was impaired significantly (Fig. 2B, 3).
- Complementation of the  $\Delta so2426$  mutant restored growth (Fig. 4A) and Cr(VI) reduction activity.
- Interestingly, the *so3585* mutant was able to reduce Cr(VI) at a substantially faster rate compared to the WT strain (Fig. 3).
- We are currently completing microarray profiling studies of the *so2426* mutant strain to gain some insight into the regulon of this response regulator. Overexpression and purification of SO2426 is also in progress.
- Future studies will focus on the purification and biochemical characterization of the putative azoreductase.

### ACKNOWLEDGMENTS

- This research was supported by the U.S. Department of Energy, Office of Science, Biological and Environmental Research programs.
- M. Thompson acknowledges support from the ORNL-UTK Genome Science and Technology Graduate School.
- We acknowledge Dr. J. Klappenbach for kind gift of pJ100 and other *E. coli* strains used in generation of mutants and K. M. Peterson for pBBR1MCS-5.
- We are indebted to Drs. Jizhong Zhou and Liyao Wu for whole-genome *S. oneidensis* MR-1 microarrays. We thank Tingyan Yan and Xueduan Liu for technical assistance in the construction of MR-1 arrays. We thank Drs. Jennifer Morrell-Falvey and Mitchell Doktycz for allowing us to use confocal and AFM microscopes.
- Oak Ridge National Laboratory is managed and operated by the University of Tennessee-Battelle, L.L.C., under contract DE-AC05-00OR22725 with the U.S. Department of Energy.
- Project web site: [http://compbio.ornl.gov/shewanella\\_chromium\\_stress/](http://compbio.ornl.gov/shewanella_chromium_stress/).